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PROVISIONAL SPECIFICATION

Invention Title:

Plants and feed baits for controlling damage from feeding insects

The invention is described in the following statement:

Field of the Invention:

The present invention relates to the problem of damage caused to plants (e.g. crop plants) from feeding insects such as lepidopterans and coleopterans. More particularly, the present invention relates to a plant capable of expressing a fusolin and/or fusolin-like protein in a tissue or tissues susceptible to damage by feeding insects. Fusolin and fusolin-like proteins ingested by feeding insects may reduce damage to the plant by inhibiting feeding, growth and/or development of insects and by subsequently also increasing susceptibility to infection by insenct pathogens.

Background of the Invention:

Entomopoxviruses (EPVs) are insect-specific members of the family *Poxviridae* (Murphy *et al.*, 1995) that collectively infect hosts such as caterpillars, beetles and locusts (Arif, 1995). Like other members of the poxvirus family (i.e., the chordopoxviruses; ChPVs), EPVs have large double-stranded DNA genomes, produce complex virions, and replicate in the cytoplasm of infected cells (Moss, 1996). While these and other molecular characteristics confirm their poxvirus affinities (Osborne *et al.*, 1996), other notable traits differentiate EPVs from ChPVs, and ally them instead with unrelated groups of insect-infecting viruses. Foremost among these traits is production of the distinctive proteinaceous structures known as spheroids and spindle bodies.

Spheroids develop in the cytoplasm of EPV-infected cells at the site of viral morphogenesis, and when mature, occlude large numbers of infectious virions (Goodwin *et al.*, 1991). They are the agent of horizontal transmission of EPVs, and while their major constituent matrix protein (spheroidin; Hall & Moyer, 1991) has no known homologue outside the taxon, the bodies themselves are assumed to protect virions from detrimental environmental factors such as desiccation and exposure to u.v. light. In this respect they are functionally analogous to the polyhedral bodies which occlude virions of members of the baculovirus family and the cytoplasmic polyhedrosis group of reoviruses.

Most EPVs also encode and produce a protein known as fusolin, which has been shown to be the major constituent of structures known as spindle bodies (SBs; Dall *et al.*, 1993); these structures have been described from many, but not all,

members of EPV genera A and B that infect caterpillars and beetle larvae (Goodwin *et al.*, 1991). In the *Heliothis armigera* EPV (HaEPV)(Fernon *et al.*, 1995) the fusolin protein has a calculated M_r of 40132, and the mature form of the protein has an apparent size of 50K when analysed by SDS-PAGE (Dall *et al.*, 1993). The protein has been found to accumulate in vesicular structures derived from cellular endoplasmic reticulum, where it eventually aggregates and crystallises into SBs (Lai-Fook & Dall, in preparation).

Genes encoding homologues of the fusolin protein, in this context known variously as "gp37", "37K protein", "SLP" (spindle-like protein), etc., have also been described from a number of nucleopolyhedrosis (NPV) baculoviruses, including the *Autographa californica, Bombyx mori, Choristoneura fumiferana, Lymantria dispar* and *Orgyia pseudotsugata* NPVs (AcMNPV, BmMNPV, CfMNPV, LdMNPV and OpMNPV, respectively; Wu and Miller 1989; Vialard *et al.*, 1990; Gross *et al.*, 1993; Maeda, 1994; Thiem, 1995; Liu and Carstens, 1996). In some of these (e.g., OpNPV; Gross *et al.*, 1993) the protein has been localised to cytoplasmic aggregates reminiscent of SBs. In a number of other instances virally-induced structures highly reminiscent of SBs in appearance (spindle-like bodies, SLBs) have been described in ultrastructural studies of NPV-infected material (e.g., from *Cadra cautella* NPV, Adams and Wilcox 1968; see also Adams and McClintock, 1991; Cunningham, 1971; Huger and Kreig, 1968; Smirnoff, 1970), although the protein constituents of these bodies have not been characterised.

As shown in Figure 1(a), all members of the fusolin group of proteins, irrespective of their viral family of origin, are united by an absolute conservation of amino acid residues at a number of positions in their sequences, in particular in the N-terminal and central regions of the molecule. These conserved residues include the presence of an HGY (standard one letter amino acid code) motif near the N-terminus of the deduced protein sequence, and FGDK, FCPT, YVRWQR and GEGFYNC sequences elsewhere within the deduced amino acid sequence. As shown in Figure 1(b), the deduced amino acid sequences of the proteins described in Figure 1(a) show identity levels of 37% to 95% in pairwise comparisons using the GCG Gap algorithm at default parameter settings. The observed conservation of sequence elements, like that of the protein's intracellular location, as previously described, suggests that all members of the group also share a common role in the

cycle of virus infection and replication, perhaps in influencing the relationship of the viruses with their hosts (Sriskantha *et al.*, 1997).

Through experiments conducted using recombinant EPVs wherein the fusolin gene has been replaced with a β-galactosidase marker (i.e., to render the recombinant EPVs fusolin negative [fus$^{(-)}$]), the present inventors have been able to determine that fusolin enhances the infectivity of the homologous EPV virus. This result is similar to that reported by Xu and Hukuhara (1992, 1994) who showed that a factor in the spheroids of *Pseudaletia separata* EPV (PsEPV), later identified as fusolin (Hayakawa *et al.*, 1996), was capable of enhancing the infectivity of a heterologous nucleopolyhedrosis virus (*P. unipunctata* NPV). However, in addition, the present inventor's experimentation with fus$^{(-)}$ recombinant EPVs has led to the unexpected discovery that fusolin can affect the feeding, growth and/or development of insects. As a result, it has been realised that fusolin and/or fusolin-like proteins may be advantageously used in strategies designed to reduce damage caused to plants by feeding insects.

Summary of the Invention:

Thus, in a first aspect, the present invention provides a plant transformed with at least one polynucleotide molecule comprising a nucleotide sequence(s) encoding a fusolin and/or fusolin-like protein(s) operably linked to a suitable promoter sequence(s), wherein said transformed plant expresses the fusolin and/or fusolin-like protein(s) in, at least, plant tissue or tissues susceptible to damage by feeding insects.

In a second aspect, the present invention provides a feed bait composition comprising fusolin and/or fusolin-like protein(s) together with an edible carrier.

Detailed disclosure of the Invention:

As mentioned above, the invention provides a plant capable of expressing fusolin and/or a fusolin-like protein(s) in tissues (e.g. leaf tissue or a product tissue such as fruit tissue) susceptible to damage by feeding insects. Thus, along with plant tissue, when feeding insects feed on a plant according to the invention, they will ingest the expressed fusolin and/or fusolin-like protein(s). Since fusolin and fusolin-like proteins inhibit feeding, growth and/or development of insects and

potentially increase susceptibility to infection from insect pathogens (and thereby insect death), ingestion of the fusolin and/or fusolin-like protein(s) by feeding insects may reduce further damage to the plant. In addition, it is believed that inhibiting the feeding, growth and/or development of insects also increases the likelihood of insect death resulting from, for example, adverse environmental conditions, predators and chemical and other biological agents (e.g., pathogenic bacteria).

The plant according to the invention may be any plant of agricultural, arboricultura, horticultural or ornamental value that is susceptible to damage by feeding insects. Preferably, the plant is selected from plants of agricultural value such as cereals (e.g.; wheat and barley), vegetable plants (e.g.; tomato and potato) and fruit trees (e.g., citrus trees and apples). Other preferred plants include tobacco and cotton.

The polynucleotide molecule(s) comprising a nucleotide sequence encoding a fusolin and/or fusolin-like protein(s) operably linked to a suitable promoter sequence(s), may be any polynucleotide molecule(s) that may be stably segregated and retained in daughter cells. Preferably, the polynucleotide molecule(s) is stably integrated into a non-essential site within the plant genome (as may be achieved by the well known technique of homologous recombination).

The polynucleotide molecule(s) may comprise a nucleotide sequence encoding one or more fusolin and/or fusolin-like protein(s).

Preferred fusolin proteins include those from HaEPV, *Pseudaletia separata* EPV (PsEPV), *Choristoneura biennis* EPV (CbEPV) and *Dermolepida albohirtum* EPV (Stone River isolate; $DaEPV_{SR}$; Dall *et al*, unpublished. Most preferred is the fusolin from HaEPV such as is described in the present applicant's Australian Patent No. 668734, the disclosure of which is to be regarded as incorporated herein by reference.

By the term "fusolin-like protein" we refer to all insect virus proteins and functional fragments thereof which are capable of inhibiting feeding, growth and/or development in at least one insect species, and which preferably also increases susceptibility in at least one insect species to infection from at least one pathogen virus (e.g. a virus). As such, the term includes all proteins (and functional fragments thereof) from entomopoxvirouses (EPVs),nucleopolyhedrosis (NPV)

baculoviruses, and all other insect viruses that demonstrate ≥ 35% amino acid sequence identity to the HaEPV fusolin protein and which include the following partial amino acid sequences: HGY, FGDK, FCPT, YVRWQR and GEGFYNC. Preferred fusolin-like proteins include those from AcMNPV, BmMNPV, CfMNPV, LdMNPV and OpMNPV.

Where the plant expresses more than one fusolin and/or fusolin-like protein, the plant may be transformed with a single polynucleotide molecule such that the proteins are expressed from single or multicistronic messenger RNA. Alternatively, the proteins might be expressed from two or more polynucleotide molecules co-transformed into the plant.

The suitable promoter sequence(s) for the expression of the nucleotide sequence(s) encoding the fusolin and/or fusolin-like protein(s), may be selected from any promoter sequence which is functional in plants. Preferred promoter sequences include those from plants and plant viruses and viroids. Particularly preferred promoter sequences include the cauliflower mosaic virus (CaMV 35S promoter element , and promoter elements from the sub-clover stunt virus (SCSV).

The plant according to the invention may also express a heterologous toxin or other agent that is deleterious to insects. For example, the plant may also express a *Bacillus thuringiensis* δ-toxin, an insect neurohormone, or an antisense RNA or ribozyme targeted against an essential cellular function. The heterologous toxin or deleterious agent may be encoded by a nucleotide sequence (operably linked to a suitable promoter sequence) borne on the polynucleotide molecule(s) encoding the fusolin and/or fusolin-like protein(s) or may be borne on a further polynucleotide molecule which has been co-transformed into the plant.

Transformation of the plant with the polynucleotide molecule(s) may be achieved by any of the well known methods in the art including *Agrobacterium* transformation and electroporation.

As will be appreciated, the benefits achieved by expressing fusolin or fusolin-like proteins in plants might also be achieved by producing feed baits comprising fusolin and/or a fusolin-like protein. Thus, feed bait compositions according to the invention comprise fusolin and/or fusolin-like protein(s) together with an edible carrier.

The feed bait compositions may be in a liquid or gel form, but more preferably are in a solid form. The fusolin and/or fusolin like protein may comprise 0.15 TO 15.0% (by weight) of the composition. In addition to the fusolin and/or fusolin-like protein and the edible carrier, the feed bait composition may comprise a pheromone(s) or other chemical attractant to insects. For liquid formulations the edible carrier may be selected from ingredients such as milled clays or plant materials, molasses or raw sugar, or micro-organisms such as yeasts or other fungi, algae or bacteria. For solid feed bait compositions, the edible carrier may be selected from ground or fragmented plant material and other materials as described above processed to an appropriate form. The solid feed bait compositions may be provided as pellets and applied by casting over an area containing a plant for which protection against damage by feeding insects is desired. Liquid or gelled feed bait compositions may be applied to a plant by spraying.

The fusolin or fusolin-like protein(s) included in the feed bait composition may be isolated from natural sources or, more conveniently, produced recombinantly in, for example, microbial or cell culture.

Insects having ingested fusolin and/or a fusolin-like protein as the result of having fed on a plant or feed bait composition according to the invention may, as mentioned above, be expected to have reduced life times as a result of an increased susceptibility to adverse environmental conditions or chemical and biological agents. Accordingly, the present invention further extends to methods where a plant in accordance with the first aspect or a plant to which a feed bait composition in accordance with the second aspect has been applied, is treated with a an insecticidal chemical and/or biological agent. Suitable chemical agents include organophosphate compounds and suitable biological agents include pathogenic bacteria and insect viruses (especially *Bacillus thuringiensis* [Bt] and nucleopolyhedrosis baculoviruses). These agents may be applied by any of the well known methods in the art and, most conveniently, by spraying. Preferably, the chemical or biological agent is applied in the form of a composition comprising an acceptable agricultural carrier. Where used with a feed bait composition, it is to be understood that the feed bait composition might also be applied to the plant after or concurrently with the chemical an/or biological agent.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated component, feature or step or group of components, features or steps with or without the inclusion of a further component, feature or step or group of components, features or steps.

The invention is hereinafter described with reference to the accompanying figures and the following, non-limiting example.

Brief Description of the accompanying figures:

Figure 1.(a) Alignment of available amino acid sequences of fusolins and homologous proteins. Amino acid residues conserved in all sequences are indicated with asterisks. Sequences, and their sources, are as follows; HA: HaEPV, Dall *et al.*, 1993; PS: PsEPV, Hayakawa *et al.*, 1996; CB: CbEPV, Yuen *et al.*, 1991; MM: *Melolontha melolontha* EPV, Gauthier *et al.*, 1995; AC: AcMNPV, Wu and Miller 1989; BM: BmMNPV, Maeda, 1994; CF: CfMNPV, Liu and Carstens, 1996; OP: OpMNPV, Gross *et al.*, 1993; LD: LdMNPV Thiem, 1995. (b). Percentage amino acid identities of fusolin and fusolin-like sequences identified in (a), as calculated by pairwise comparisons using the GCG Gap algorithm at default parameter settings.

Figure 2. Transfer vector pEPAS3.

Figure 3. Protein constituents of wild-type and recombinant [fus$^{(-)}$] isolates of HaEPV, visualised (a) by staining with Coomassie Blue, or (b) by Western blotting with antiserum to HaEPV fusolin. Arrows indicate positions of fusolin protein.

Figure 4. Infectivity of wild-type and recombinant [fus$^{(-)}$] isolates of HaEPV for 48 hr old *Helicoverpa armigera* larvae.

Figure 5. Weight gain profiles of 48 hr old *Helicoverpa armigera* larvae after 7 days feeding on diet contaminated with wild-type and recombinant [fus$^{(-)}$] isolates of HaEPV.

Figure 6. Developmental fate of 48 hr old *Helicoverpa armigera* larvae after 21 days feeding on diet contaminated with wild-type and recombinant [fus$^{(-)}$] isolates of HaEPV.

Example:

In these studies. the transfer vector pEPAS3, which, as shown in Figure 2, contains a bacterial *lacZ* gene inserted immediately upstream of the HaEPV fusolin coding sequence, in a manner that prevents expression of the latter, was used, together with wild-type HaEPV, to produce recombinant forms of HaEPV in which fusolin production was replaced by production of the b-galactosidase marker protein. Amplified stocks of that recombinant HaEPV were subsequently found to contain forms of the virus which produced neither the b-galactosidase marker, nor the fusolin protein, as judged by the absence of SBs in preparations viewed by light microscopy. Two such variants (pp5 and pp7) were isolated by repeated plaque purification and subsequent re-amplification in insect cell cultures. Harvested preparations of cells infected with these viruses were then fed to larvae of the moth *Helicoverpa armigera*, establishing, in turn, infections in those insects. Infected insects were processed to recover the products of these infections for use in subsequent biological investigations, and preparations of virus stocks known as pp7T6 and pp7S22 (or, following a second insect passage, pp7T6/5 and pp7S22/13) were ultimately selected for more detailed characterisation.

Stocks of the wild-type clonal isolate wt#2/011293 (Osborne *et al.*, 1996), which was used as the parental form for production of the original b-galactosidase expressing recombinant, were carried in parallel through plaque purification, re-amplification, and feeding to/ recovery from *H. armigera* insect hosts. Selected lines from these stocks (2C1 and 2D8, or 2C1/11 and 2D8/17) served as controls in the investigations described below.

Light microscopy and scanning electron microscopy was used to examine the composition and morphology of preparations of stocks pp7T6, pp7S22, 2C1 and 2D8. As expected, preparations of the wild-type viruses 2C1 and 2D8 were observed to contain both virus spheroids and SBs, while preparations of the recombinants pp7T6 and pp7S22 were observed to contain only spheroids. The spheroids of all four stocks appeared to be morphologically identical.

The molecular composition of preparations of these virus stocks was examined using the standard laboratory protocols of SDS-PAGE and Western blotting (see, e.g., Sambrook *et al.*, 1989). As shown in Figure 3(a), Coomassie Blue staining of the separated protein constituents of all four preparations showed a

prominent band of about 115 kDa, corresponding to the major spheroid matrix protein (spheroidin; Hall & Moyer, 1991; Sriskantha *et al.*, 1997), and numerous other less intense bands apparently common to each. Preparations of the two wild-type stocks also showed a band of protein with a mobility of about 50 kDa, (Figure 3[a], arrow) corresponding to the monomeric form of the fusolin protein (Dall *et al.*, 1993), that was not apparent in preparations of pp7T6 and pp7S22. A polyclonal antiserum to HaEPV fusolin protein (Dall *et al.*, 1993) and Western blotting protocols were then used to further characterise these virus stocks. As shown in Fig. 3(b), both preparations of wild-type virus produced very prominent immuno-reactive bands at a position corresponding to a molecular weight of about 50kDa (arrow), which, as expected, were not apparent in preparations of the two fusolin negative [$fus^{(-)}$] recombinant forms.

One wild-type isolate (2D8) and one recombinant (pp7T6) were then selected for more detailed biological characterisation. Individually housed 48 hour old *H. armigera* larvae were exposed to a range of quantities of each of the viruses by placing them on artificial diet spread with aliquots of virus dilution series. Seven days later ("post-infection"; 7 dpi) each larva was weighed and at 21 dpi all larvae were collected, their developmental stage was recorded, and their status with respect to viral infection (i.e., infected or uninfected) was determined by examination of fat body smears by light microscopy. In all instances, larvae that died at or before 7 dpi were excluded from the assay, while those that were dead at 21 dpi were considered to be positive (i.e., infected).

As shown in Figure 4, these experiments demonstrated that the wild-type virus isolate 2D8 was substantially more infectious than the $fus^{(-)}$ recombinant pp7T6, with the former having an estimated IC_{50} (this being the quantity of virus required to infect 50% of exposed larvae) of 0.2 spheroids/mm^2 diet (sph/mm^2), while for pp7T6 it was 35 sph/mm^2. Results of less detailed investigations with virus isolates 2C1 and pp7S22 were also consistent with these results.

Further analysis of the results has revealed, in addition, another fusolin associated phenomenon which has not previously been recognised, namely, that the presence of fusolin is associated with retardation of the rates of growth and development of exposed insect larvae. Thus, Figure 5 shows mean weights of infected insects only, taken at 7 dpi, and calculated as a proportion of the weight of

uninfected larvae from the same cohort, i.e. as a % of the weight of experimental controls. As can be observed, when the results were analysed in this manner it was clear that in the presence of fusolin, larval weight gain was much reduced. This analysis thus makes allowance for the previously described observation, i.e., that the presence of fusolin enhances virus infectivity, and further shows that when intrinsic infectivity of a particular dose is used as the basis of comparison, this previously unrecognised effect of fusolin on insect growth can be observed.

Similarly, and as shown in Figure 6, when the developmental fate of those same infected insects, now pooled in three "categories" of infection rates, was assessed at 21 dpi, a much reduced proportion of larvae was observed to proceed to pupation in samples exposed to preparations of the wild type virus containing the fusolin protein.

As a direct consequence of the observations described above, it can now be predicted that strategies designed to effect oral ingestion of fusolin by feeding insects will inhibit feeding, growth and/or development of insects. Such strategies may therefore be of significant value with respect to limiting losses to commodity materials that result from insect feeding activity. That is, it can be appreciated that small insects cause less feeding damage to plants than do large ones, and that retarding the growth and/or development of insects will increase the time-span during which factors such as adverse environmental conditions, predators, and/ or artificially applied chemical and biological agents may effect their control. In addition, it is widely recognised that early instar (i.e., smaller) insects are intrinsically more susceptible to infection with, or the activity of, a variety of chemical and biological control agents such as the bacterium *Bacillus thuringiensis* ("Bt").

Further experimentation is presently underway to demonstrate the utility of the observations in the following manner. Oligonucleotide ("oligo") primers (FSexp1, 5'ATAATGAATAAATTCTATTA, and FSexp2, 5'CTCCCGAAATTAATAATTTTTG) have been designed for use in the polymerase chain reaction, and used to amplify a fragment of DNA containing the entire nucleotide sequence encoding the fusolin protein of wt#2/011293 HaEPV. The amplicon produced in this manner has been be cloned into the plasmid vector pDH51 (Pietrzak *et al.*, 1986), between nucleotide sequences corresponding to the

cauliflower mosaic virus (CaMV) 35S promoter and the CaMV 35S poly-A terminator element. The resultant vector (pFus1) will now be electroporated into protoplasts of *Nicotiana plumbaginifolia* (tobacco), where it is expected that it will express the fusolin protein. These protoplasts will then be used in feeding trials with *H. armigera* larvae, in order to conclusively demonstrate the effect of oral ingestion of fusolin on larval growth and development. In a second experiment, the portion of the plasmid vector pFus1 comprising the promoter, fusolin-encoding, and terminator sequences, as described above, will be excised with the restriction endonuclease *Eco*RI, and inserted into a binary vector such as pART27 (Gleave, 1992). This manipulated binary vector will then be used in *Agrobacterium tumifaciens*-mediated transformation protocols, to produce transformed plants of, e.g., *Nicotiana plumbaginifolia*, which, in turn, will further be used to demonstrate the effect of ingestion of fusolin in plant-derived diet on growth, development and susceptibility to various chemical and biological control agents.

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It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this 10th day of March 1999

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
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Figure 1(a). **Fusolin homologues from EPV and NPV sources**

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HA   MNKFYYICIY INILYVCVSG HGYMTFPIAR QRRCSVRGGQ WWPPNGDGIT DTMCRAAYQN VYNKVLNQY. NDPQEAATAA QYMFQQDNEY AALAGPDYTN
PS   MNKFYYICLY INILYVCVNG HGYMTFPIAR QRRCSVRGGQ WWPPNGDGIT DTMCRAAYKN VYNKVLNQY. NDPQEAATAA QYMFQQDNEY AALAGPDYTN
CB   MNKLILISLI ASLYQVEVDA HGYMTFPIAR QRRCSAAGGN WYPVGGGGIQ DPMCRAAYQN VFNKVLNSNG GDVIDASEAA NYMYTQDNEY AALAGPDYTN
MM   ..MFIKILPI LILFLDYVSG HGYITFPIAR QRRCNVQGGF WWPPGGSGIP DPMCRAAYQN VYNKVLQQ.G GTIDQAASAA QYMFQQDNEY AALAGPNYLD
AC   .MIALLIALF AAIHAPAVRS HGYLSVPTAR QYKCFKDGNF YWPDNGDNIP DAACRNAYKS VYYK.YRALD LESGAAASTA QYMFQQYMEY AAVAGPNYDD
BM   .MIALLIALF AVIHAPAVRS HGYLSLPTAR QYKCFKGGNF YWPHNGDKIP DAACRNAYKS VYYK.YRALD LESGAAAATA QYMFQQYMEY ASVAGPNYDD
CF   .MYKLGVALF ALFLAPAVRS HGYLSVPVAR QYKCFRDGNF WWPNNGDNIP DEACRNAYKK VYYK.YRAIN VPSQEAASAA QYMFQQYTEY AALAGPNYLD
OP   .MYKLCAVLF A.LAVPAVRP HGYLSTPVAR QYKCFADGNF YWPDNGDGVP DEACRNAYKK VFHR.YRAVG APPGEAAAAA QYMFQQYAEY AAVAGPNYRD
LD   MFIWATICAV AAALATTADA HGYLSRPAAR QFKCFRDGRF WWPETGEDIP DEACRRAYRT VYAK.YRSSG ESGGVAANAA QYMFQQYFEY AALAGADYQN
                           ***   * ** *  *   *     *  *     *  ** **   *               *   *  *** *  ** *   **  *

HA   LCNLQQNVVP NNLCAAGADD WDVVPFGDKS GMDLP..GNW VPTVIPLDSN HQSSVALELE FCPTAVHDPS YYEVYITNSG FNVHTDNVVW GNLELI.FND
PS   LCNLQQNVVP SNLCAAGAAD WDVVPFGDKS GMDLP..GNW VPTVIPLSSN HQSSVALELE FCPTAVHDPS YYEVYITNSG FNVHTDNVVW GNLELI.FND
CB   ICHIQQRVVP SYLCAAGASD WSIRPFGDKS GMDLP..GSW TPTIIQLSDN QQSNVVMELE FCPTAVHDPS YYEVYITNPS FNVYTDNVVW ANLDLI.YNN
MM   QNHIRNNVVP NYLCAAHATT WRIRPFGDKT GMDVS..GSW TPTVIPLQDN TVSTVPIEFE FCPTAIHEPS FFEIYITVPS FNVYTDQVTW QQLINI.FTG
AC   FDLIKQRVVP HTLCGAGSND RN.SVFGDKS GMDEPFNNWR PNTLYLNRYQ PVYQMNV..H FCPTAIHEPS YFEVFITKSN WD.RRNPITW NELEYIGGND
BM   FDLIKQRVVP HTLCGAGSND RN.SVFGDKS GMDEPFNNWK PNTLYLNLYQ PVYRMNV..H FCPTAIHEPS YFEVFITKSN WD.RRNPITW NELEYIGGND
CF   FDMVKRDVVP HTLCGAASND RA.ALFGDKS GMDEPFYNWR PDVLYMNRYQ NSYPMDV..H FCPTAIHEPS YFEVFVTKST WD.RRNPITW NELEYIGGNN
OP   LELVKREVLP HTLCGAAAND RH.ALFGDKS GMDEPFHNWR PDVLYVNRYQ RAHSFNV..H FCPTAVHEPS YFEVYVTKFT WD.RRSPVTW NELEYIGGNG
LD   LEHVQENVVA ADLCAAGASD RS.RPFGDKS GIDEPFDDWR ADTLYPRADE SASSRAVALH FCPTTVHEPS FFQIFISREA YN.YTRKLTW RDLELVENET
            *     ** *          ****  * *                              ****  * **                     *   *

HA   TVPLRPKSST STCNANPNVY RFTVSIPVRP AQFVLYVRWQ RIDPVGEGFY NCVDMAFDYA AGPSEEDVIY PD........ .......... ..YEAPGQNA
PS   TVPLRPKAST STCTANSNVY RFTVSIPVRP AQFVLYVRWQ RNDPVGEGFY NCVDMDFDYE AGPPEDDIIY PD........ .......... ..EESPGDSG
CB   TVTLRPKLPE STCAANSMVY RFEVSIPVRP SQFVLYVRWQ RIDPVGEGFY NCVDMKFKYS EGPDEEDIIE PE........ .......... ..YEVDNEAE
MM   PIPLVQRRPD SQCNAHNLVY RTTVGIPVRQ TQFVLYVRWQ RNDPVGEGFY NCADVIFAHR LGINEEDKIR PPKMKCKGND KDCYKHHHRH NRYENDYENN
AC   SN.LIPNPG. DSLCDNSLVY SIPVVIPYRS NQFVMYVRWQ RIDPVGEGFY NCADLVFETL DDECRYAQMA KVVRSQLQKH KLD....... ..........
BM   SD.LIPNPG. DPLCDNSLVY SIPVVIPYRS NQFVMYVRWQ RIDPVGEGFY NCADLVFETL DDECRYAQMA KVVRSQLQKH KLD....... ..........
CF   SG.LVPNPG. DPLCDSNQIY SIPVSVPYRS GQFVMYVRWQ RIDPVGEGFY NCADLVFESD DDECRYARAA KAVRDQLRNQ DQDRCDDCEG DDAEDVGVGD
OP   SG.LVPNPG. DAFCASGQLY SIPVSVPYRP GPFVMYVRWQ RIDPVGEGFY NCADLVFGTE NDECRYARAA KAVRDQLRQQ NL........ ..........
LD   PARLIANDGT DENCASPSVY ALEARVPLRP GKFVLYVRWQ RRDVAGEGFY NCADVTFG.. RQQSRNARSK SR
        *                *       * *    ** ***** * *  ***** ** *  *

HA   .YTCHANRNK YG.GNYENTI DEDKYQAQLD ESIKSRYDKY SRHK..GGKF GQKQCNGNKH HYNKYTKYYN QNYKNNKNY
PS   .YTCHENRNR HHYGIYENTI DDDKYQAQLT DSIKSRYDKY SRHK..GGRY GKGTCKGNKN YYNKYTKYYN QNYKNNKNY
CB   CFAYRTNSG. ...NVNVNPL QENKYMAYAN KAIR....NI NTHS..NG.. ....CSRNRN NKNNYNKYYS KTYNYNQNRK
MM   YENYENYENN YENNYENNYE NNYEYEYEYD RNNREHYHKC KHHSCMQHNY YERQYNTKDF NYVEWNDDYS DYIEIIQDNR DMCDSTTKCC YKK
AC   .....ARIDH NDEESCWRAR KSNYSSFFNP GF
BM   .....ARIDH NDEKSCWRAH KSNC
CF   GGAENGNGDN DDEESCVRTR RRGYYNTHDN RNRRHQQHRD HNRGYRRGNG RSRRYGTHGK SNNDPTVRET IDEL
OP   ...CNDCVEA GPQESCAPTR PQ........ .......... .....RRAHN YLRRGGAHDQ QADGASVRET IDEL
LD
```

djd/c/patents/fusexp/fsfig1

Figure 1(b)

	PS	CB	MM	AC	BM	CF	OP	LD
HA	90	63	52	42	43	38	39	44
PS		62	51	43	43	39	38	44
CB			53	42	42	37	39	42
MM				40	40	39	37	44
AC					95	76	65	48
BM						72	65	47
CF							74	49
OP								52

Figure 2

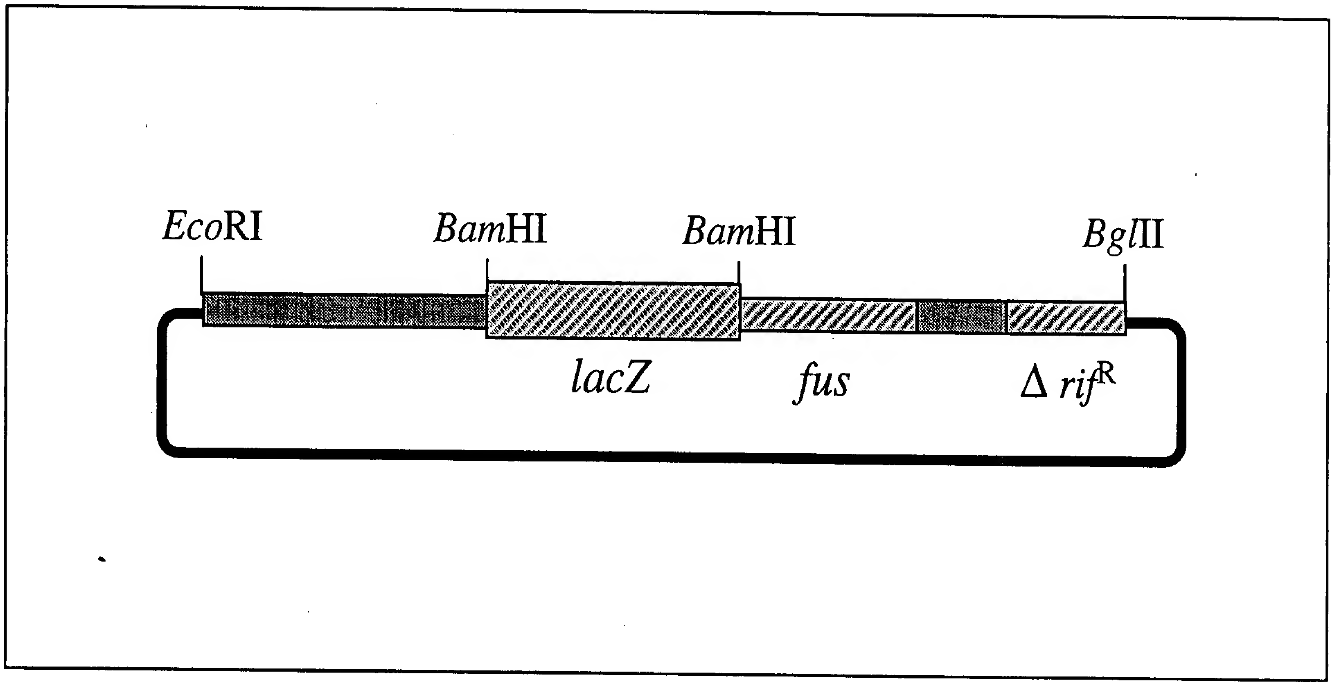

Figure 3

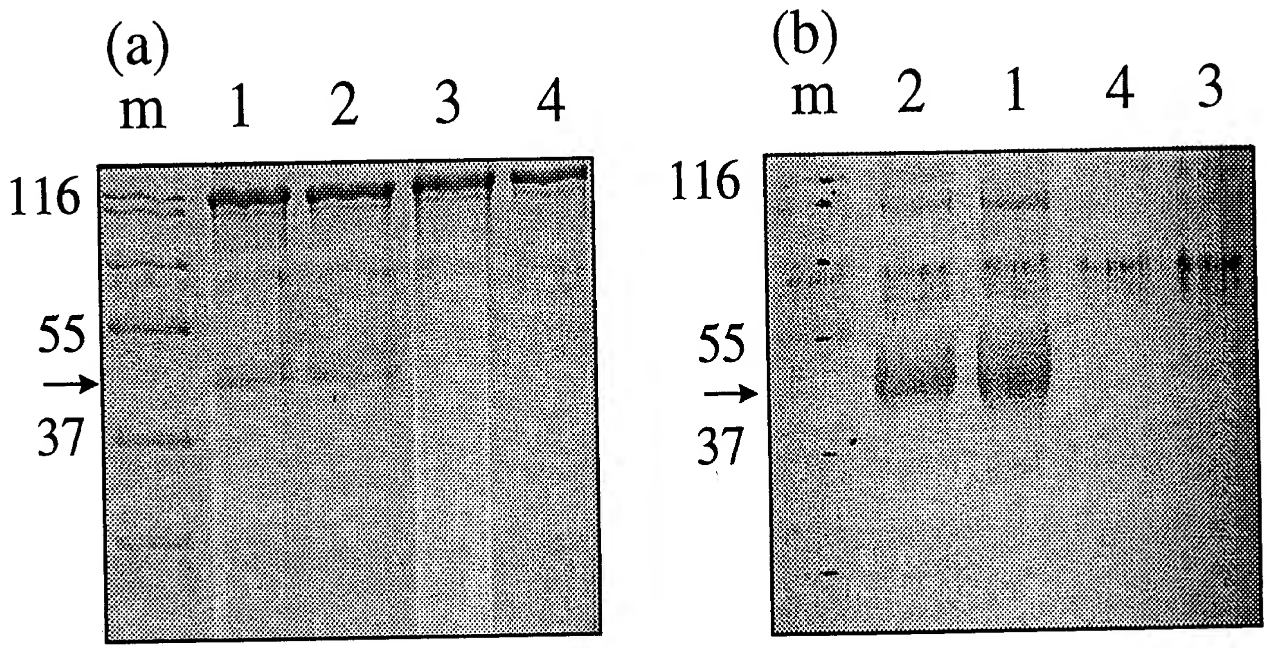

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Figure 4

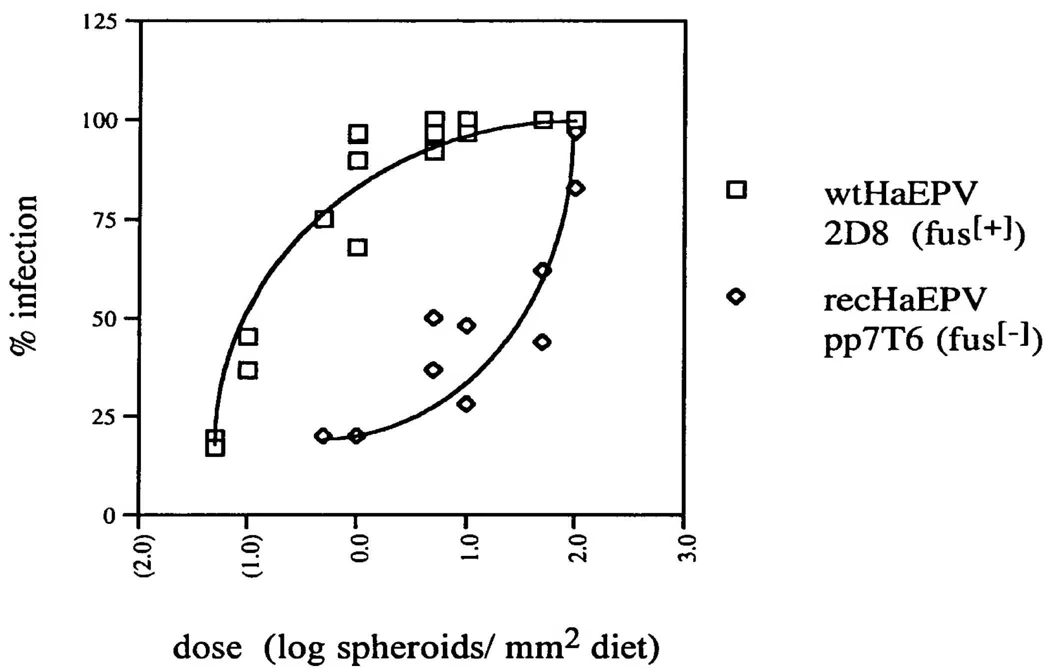
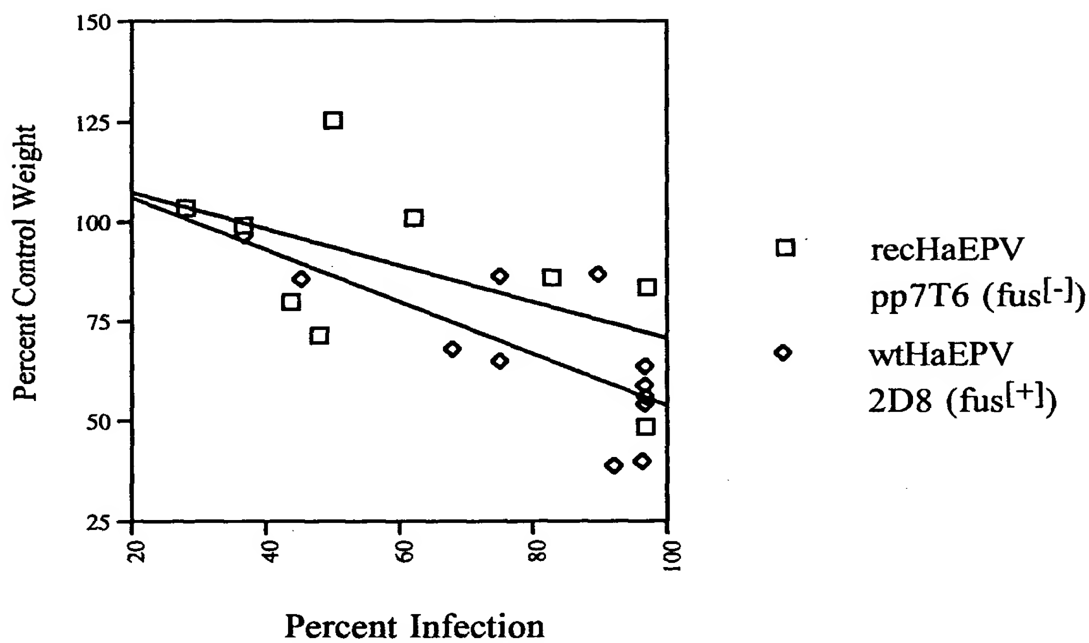
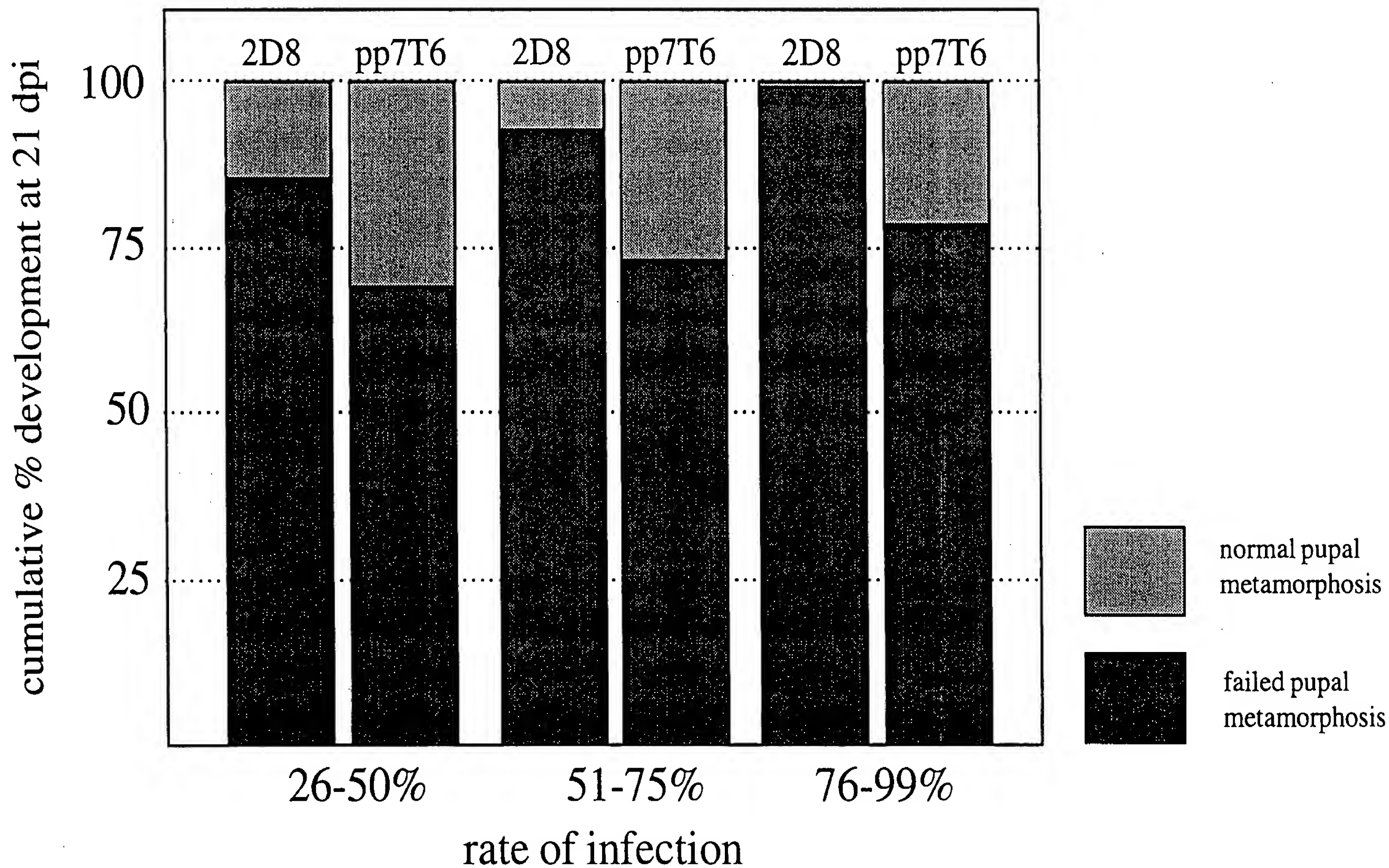

125
100
75
50
25
0
% infection
(2.0)
(1.0)
0.0
1.0
2.0
3.0
dose (log spheroids/ mm2 diet)
wtHaEPV
2D8 (fus[+])
recHaEPV
pp7T6 (fus[-])

Figure 5



150
125
100
75
50
25
Percent Control Weight
20
40
60
80
100
Percent Infection
recHaEPV
pp7T6 (fus[-])
wtHaEPV
2D8 (fus[+])

Figure 6


cumulative % development at 21 dpi
100
75
50
25
2D8
pp7T6
2D8
pp7T6
2D8
pp7T6
26-50%
51-75%
76-99%
rate of infection
normal pupal metamorphosis
failed pupal metamorphosis